APPENDIX A

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Docket No.: UMY-055
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Michael P. Czech *et al.*

Application No.: 10/735461 Confirmation No.: 3119

Filed: December 11, 2003 Art Unit: 1635

For: METHOD OF INTRODUCING siRNA INTO ADIPOCYTES Examiner: R.A. Schnizer

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 CFR §1.132

Dear Sir:

We, Michael P. Czech, Qiong L. Zhou, and Zhen Y. Jiang, are named inventors in the above-identified application and make this declaration in support thereof, and particularly in response to the February 22, 2007 Office Action. I, Michael P. Czech, am Professor and Chair of the Program in Molecular Medicine at the University of Massachusetts Medical School. I, Qiong L. Zhou, am an Instructor in the laboratory of Michael P. Czech at the University of Massachusetts Medical School. I, Zhen Y. Jiang, am a Research Assistant Professor in the laboratory of Michael P. Czech at the University of Massachusetts Medical School.

(1) We understand that the Examiner has maintained the rejection of claims 27, 44-48, 50, 51, 56-59, 79, and 81-83 under 35 U.S.C. § 103(a) as being unpatentable over Al-Hasani *et al.* (*J. Biol. Chem.* 273(28):17504-17510, 1998) in view of Clancy *et al.* (US20030087259); additionally in view of Paquereau *et al.* (*Anal. Biochem.* 204(1):147-151, 1992) for claims 38-43, 84, and 85; additionally in view of Standaert *et al.* (*J. Biol. Chem.* 272(48):30075-30082, 1997) for claim 49; and additionally in view of McSwiggen *et al.* (U.S. Patent No. 7,022,828) for claims 52-55.

(2) Al-Hasani *et al.* does not describe the transfection or electroporation of adipocytes with siRNA. Rather, Al-Hasani *et al.* describes the transfection of adipose cells with ***DNA and DNA expression plasmids*** in order to characterize the mechanism of GLUT4 endocytosis by overexpressing a dominant-negative mutant of dynamin-1 in rat adipose cells.

Clancy *et al.* teach diagnostic assays for detecting bone and cartilage formation and therapeutic methods for treating disease and disorders related to bone and cartilage formation or resorption. Clancy *et al.* teach siRNAs as potential agents for "blocking or reducing the expression of a gene or the activity or level of the encoded polypeptide that is modulated, *e.g.*, upregulated, during normal bone or cartilage formation" (see *e.g.*, para. 0239)).

There is no basis in Al-Hasani *et al.* in view of Clancy *et al.* for providing any reasonable expectation of success in electroporating adipocytes with siRNA, as claimed in the present invention, for the following reasons:

(a) One of ordinary skill in the art would not have been motivated to substitute the ***DNA plasmids*** transfected in Al-Hasani *et al.* with the ***siRNAs*** disclosed by Clancy *et al.* as an agent capable of blocking gene expression. The mere fact that Clancy *et al.* lists siRNAs and dominant negative mutants as potential gene blocking compounds in a more extensive list of gene blocking compounds, *e.g.*, antisense molecules, ribozymes, triplexes, aptamers, does not arise to the level of a motivation to select one specific member from the recited antagonist list for use in the featured methodology.

(b) One of ordinary skill in the art one would not have had a reasonable expectation that a substitution of the DNA plasmids of Al-Hasani *et al.* with the siRNAs disclosed by Clancy *et al.* would result in success. Specifically, it was well known in the art at the time of filing that electroporation of DNA into adipocytes only leads to the successful expression of DNA in only a small minority of the adipocytes (***approximately 1-10%***[1]). In contrast, in order for siRNA to successfully silence the gene of interest, *i.e.*, mediate RNA interference, as currently claimed, it is required that virtually all of the adipocytes (***approximately 100%***) take up functional siRNA. ***Since the successful electroporation of DNA into adipocytes is typically less than 10% efficient, it would <u>not</u> have been obvious to one of ordinary skill in the art at the time of filing of the instant invention that electroporation of siRNA into adipocytes would be nearly 100% efficient***[2]***.***

[1] See, *e.g.*, page 40, lines 15-17 of the instant specification.

[2] Using labeled siRNA, Figure 1B, left panels, and Example 2, page 40, lines 1-17, of the specification demonstrate that the electroporation of siRNA into adipocytes was, unexpectedly, nearly 100% efficient.

A skilled artisan would have had an appreciation of these significant differences and would not have reasonably expected that mere substitution of the siRNAs of Clancy *et al.* for the plasmid DNAs transfected in Al-Hasani *et al.* would be successful.

(c) The secondary references of Paquereau *et al.*, Standaert *et al.*, and McSwiggen *et al.* fail to cure the deficiencies of the Al-Hasani and Clancy references. Specifically, Paquereau describes the transfection of ***hepatocyte cells with DNA***, Al-Hasani is describes the study of insulin stimulation in glucose transport by transfection of rat adipocytes ***with plasmid DNA***, and McSwiggen teaches the general use of modified siRNA oligonucleotides which modulate the expression or function of IKK genes, in several cell types, ***none of which include adipocytes***. Thus, these secondary references fail to rectify the deficiency of teachings of the Al-Hasani and Clancy references.

(3) The art is replete with teachings which support the non-obviousness of the present invention. Following are several examples demonstrating the difficulty of transfecting adipocytes with siRNA and the successful use of the present invention to electroporate adipocytes with siRNA:

(a) As demonstrated in Appendix A, in 2006 Robinson *et al.* state that "adipocytes are difficult to transfect, and until recently, successful siRNA transfection was achieved only via electroporation" (see, *e.g.*, page E885, second column, third full paragraph). Robinson *et al.* go on to cite a 2004 scientific publication of one of the inventors of the instant application, M. Czech, as the group which was successful in transfecting adipocytes with siRNA using electroporation.

(b) As demonstrated in Appendix B, the 2006 Panomics DeliverX Plus siRNA Transfection Kit Brochure discloses that "[t]ransfection of siRNA into differentiated 3T3-L1 adipocytes... has only been accomplished by electroporation" (see, *e.g.*, first page, left column) and specifically references the 2003 *Proceedings of the National Academy of Sciences* scientific publication by the instant inventors which corresponds to the instant patent application. This Brochure goes on to further disclose that "***adipocytes... represent one of the most difficult-to-transfect cell lines used routinely in cell biology studies***" (see, *e.g.*, page 2, right column, second full paragraph) (Emphasis added).

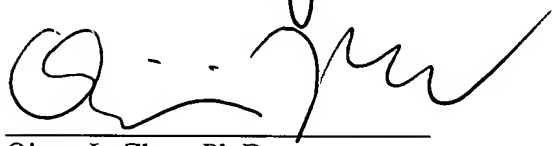
(c) As demonstrated in Appendix C, Jain discloses that "***adipocytes are fully differentiated cells with no proliferation and are thus difficult to transfect*** by either RNAi or ASO approaches" (see, *e.g.,* page 308, middle column, first paragraph) (Emphasis added).

(d) As demonstrated in Appendix D, Venugopal *et al.* disclose that "***adipocytes... proved difficult to transfect efficiently with siRNA***" (see, *e.g.,* page 17122, second column, first full paragraph) (Emphasis added).

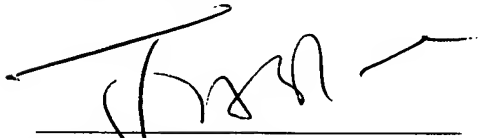
(4) In conclusion, we hereby declare that there is no basis in Al-Hasani *et al.* in view of Clancy *et al.* for providing any reasonable expectation of success in electroporating adipocytes with siRNA, as claimed in the present invention. Furthermore, the secondary references of Paquereau *et al.*, Standaert *et al.,* and McSwiggen *et al.* fail to cure the deficiencies of the Al-Hasani and Clancy references.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Michael P. Czech, Ph.D. Date July 18, 2007



Qiong L. Zhou, Ph.D. Date July 18. 2007



Zhen Y. Jiang, Ph.D. Date July 18. 2007